IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Shamay IZHAR

Confirmation No.: 8900

Patent No.: 6,852,911 B1

Application No.: 09/411,863

Patent Date: February 8, 2005

Filing Date: October 4, 1999

For: METHOD OF PRODUCING A MALE STERILE PLANT BY EXOGENIC ALLELISM

Attorney Docket No.: 85189-4300

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.322

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Patentees hereby respectfully request the issuance of a Certificate of Correction in connection with the above-identified patent. The corrections are listed on the attached Form PTO-1050, submitted in duplicate. The corrections requested are as follows:

On the title page, Item (73) Assignee, change "**Fertiseed Ltd.**, Sitrya (IR)" to -- **Fertiseeds Ltd.**, Ness-Ziona (IL) --. This change is to correct inadvertent clerical errors in completing the Issue Fee Transmittal Form PTOL-85.

In the drawings, Sheet 2, Fig. 2, step V, rows 1 and 2, change the first occurrence of "Lox" to --FRT --.

At column 16, line 36, change "(1992 XXX)" to -- (1992) --.

At column 2, line 7, change each occurrence of "LOX" to -- Lox --.

At column 21, line 4, change "his" to -- this --.

At column 22, line 25, change "cytA" to -- CytA --.

The above changes are requested merely to correct inadvertent clerical and typographical errors and do not involve the introduction of new matter.

A fee of $100 is believed to be due for this request. Please charge the required fees to Winston & Strawn LLP Deposit Account No. 50-1814. Please issue a Certificate of Correction in due course.

Respectfully submitted,

8/30/05

Date

Allan A. Fanucci, Reg. No. 30,256

WINSTON & STRAWN LLP
Customer No. 28765

212-294-3311

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO.: 6,852,911 B1
DATED: February 8, 2005
INVENTORS: Izhar

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, Item (73) Assignee, change "**Fertiseed Ltd.**, Sitrya (IR)" to -- **Fertiseeds Ltd.**, Ness-Ziona (IL) --.
In the drawings, Sheet 2, Fig. 2, step V, rows 1 and 2, change the first occurrence of "Lox" to --FRT --.
Column 16, line 36, change "(1992 XXX)" to -- (1992) --.
Column 29, line 7, change each occurrence of "LOX" to -- Lox --.
Column 21, line 4, change "his" to -- this --.
Column 22, line 25, change "cytA" to -- CytA --.

$ DAC
DFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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OIPE JC11 AUG 30 2005 PATENT & TRADEMARK OFFICE

PETITION UNDER 37 C.F.R. § 1.182

Attention: Office of Petitions
BOX DAC
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Patentee hereby Petitions Under 37 C.F.R. § 1.182 for correction of the Assignee's address on the title page of the above-identified patent for the reason stated below.

When preparing the Issue Fee Transmittal Form, the name and address of the assignee were incorrectly identified as "Fertiseed Ltd., Sitrya, Iran." It is respectfully requested that the name and address of the assignee be changed to "Fertiseeds Ltd., Ness-Ziona, Israel."

As this error was unintentional, patentees respectfully request that this Petition be granted and that a certificate of correction be issued. A Request for Certificate of Correction with Form PTO-1050 is filed concurrently herewith.

A fee of $130.00 is believed to be due for this Petition. Please charge the required fee to Winston & Strawn LLP Deposit Account No. 50-1814. A copy of this sheet is attached for accounting purposes.

08/31/2005 SZEWDIE1 00000050 501814 6852911
01 FC:1462 400.00 DA

Respectfully submitted,

8/30/05
Date

Allan A. Fanucci (Reg. No. 30,256)

WINSTON & STRAWN LLP
Customer No. 28765
212-294-3311

DC:401630.1

US006852911B1

(12) **United States Patent**
Izhar

(10) **Patent No.: US 6,852,911 B1**
(45) **Date of Patent: Feb. 8, 2005**

Fertiseeds Ltd., Ness-Ziona (IL)

(54) **METHOD OF PRODUCING A MALE STERILE PLANT BY EXOGENIC ALLELISM**

(75) Inventor: **Shamay Izhar**, Rehovot (IL)

(73) Assignee: **Fertiseed Ltd.**, Sitrya (IR)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/411,863**

(22) Filed: **Oct. 4, 1999**

Related U.S. Application Data

(60) Provisional application No. 60/151,627, filed on Aug. 31, 1999.

(51) **Int. Cl.**[7] **A01H 5/00**; A01H 5/10; C12N 15/82

(52) **U.S. Cl.** **800/303**; 800/278; 800/288; 800/287; 800/286

(58) **Field of Search** 800/278, 303, 800/288, 286, 287, 274, 298; 435/320.1, 468, 469

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,426,041	A *	6/1995	Fabijanski et al.	435/172.3
5,929,307	A *	7/1999	Hodges et al.	800/303
6,392,119	B1 *	5/2002	Gutterson et al.	

OTHER PUBLICATIONS

Stuurman et al, 1996, Plant Mol. Biol. 32:901–913.*
Kilby et al, 1995, Plant J. 8:637–652.*
Sonti et al, 1995, Plant Mol. Biol. 28:1127–1132.*
Kilby et al, 2000, J. Exp. Bot 51:853–863.*
Gidoni et al, 2001, Euphytica 121:145–156.*
Medberry, S. L. et al. "Intra–chromosomal rearrangements generated by Cre–iox site–specific recombination." 1995, Nucleic Acids Research, vol. 23, pp. 485–490.*
Golic, K. G. "Site–Specific Recombination Between Homologous Chromosomes in Drosophila." 1991, Science, vol. 252, pp. 958–961.*
Luo, H. et al. "FLP–mediated recombination for use in hybrid plant production." 2000, The Plant Journal, vol. 23, pp. 423–430.*
Qin, M. et al. "Cre recombinase–mediated site–specific recombination between plant chromosomes." 1994, Proc. Natl. Acad. Sci. USA, vol. 91, pp. 1706–1710.*
Snaith, M. R. et al. "Multiple cloning sites carrying loxP and FRT recognition sites for the Cre and Flp site–specific recombinases." 1995, Gene, vol. 166, pp. 173–174.*
Vergunst, A. C. et al. "Site–specific integration of Agrobacterium T–DNA in *Arabidopsis thaliana* mediated by Cre recombinase." 1998, Nucleic Acids Research, vol. 26, pp. 2729–2734.*
Mariani, C. et al. "Induction of male sterility in plants by a chimaeric ribonuclease gene." 1990, Nature, vol. 347, pp. 737–741.*
Lloyd, A. M. and Davis, R. W. "Functional expression of the yeast FLP/FRT site–specific recombination system in *Nicotiana tabacum*." 1994, Mol Gen Genet, vol. 242, pp. 653–657.*

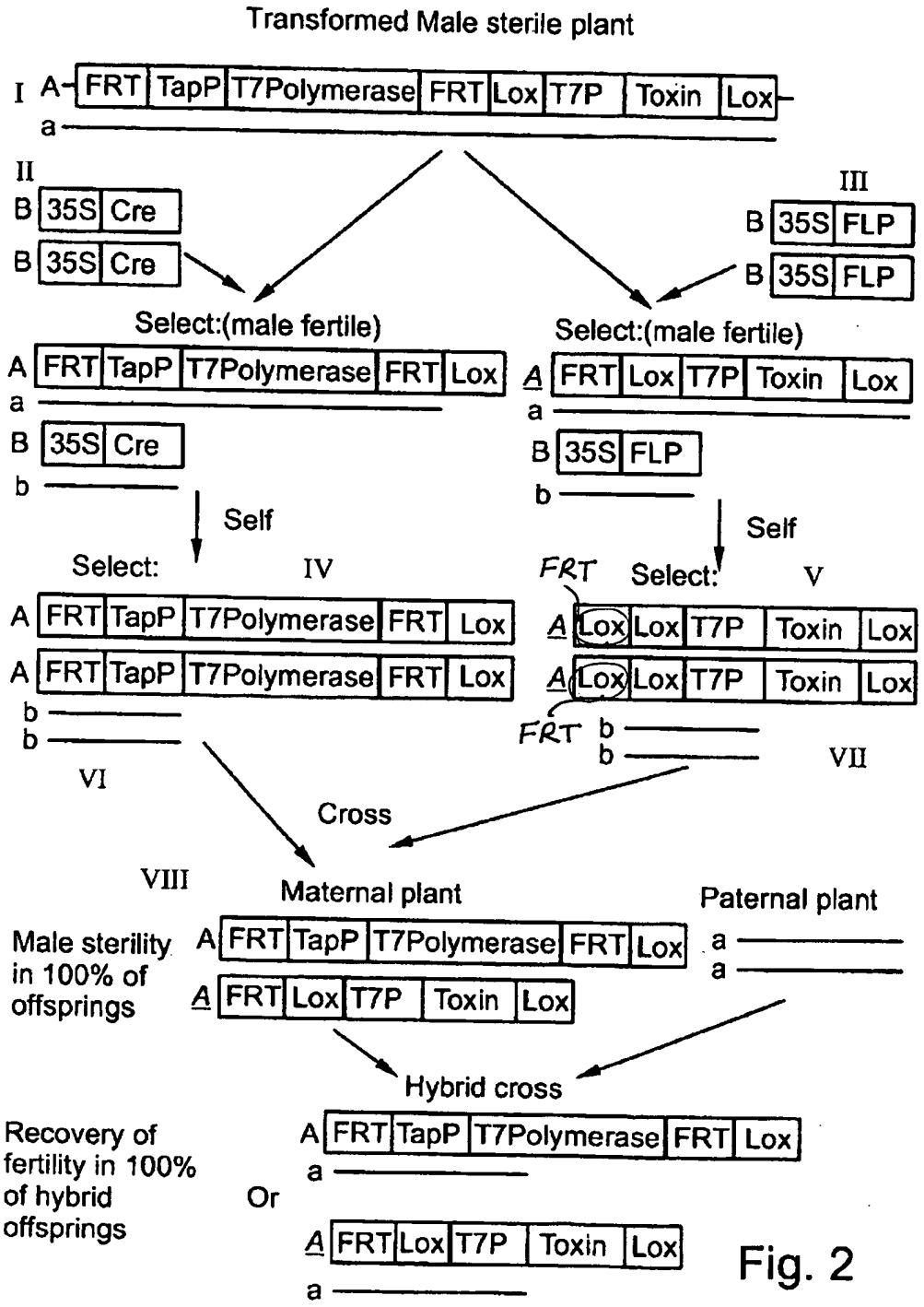
* cited by examiner

Primary Examiner—Anne Kubelik
(74) *Attorney, Agent, or Firm*—Winston & Strawn LLP

(57) **ABSTRACT**

The invention is drawn to a method of producing a male-sterile plant by using two different recombinases to generate different deletions in a transgenic construct in a plant, thereby resulting in a male-sterile plant with two different constructs in an allelic relationship to one another.

10 Claims, 2 Drawing Sheets


Transformed Male sterile plant
I
A
FRT
TapP
T7Polymerase
FRT
Lox
T7P
Toxin
Lox
a
II
B
35S
Cre
B
35S
Cre
III
B
35S
FLP
B
35S
FLP
Select:(male fertile)
Select:(male fertile)
A
FRT
TapP
T7Polymerase
FRT
Lox
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35S
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FRT
Lox
T7P
Toxin
Lox
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Select:
IV
FRT
Select:
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A
FRT
TapP
T7Polymerase
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T7Polymerase
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Lox
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T7P
Toxin
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Lox
T7P
Toxin
Lox
FRT
b
b
VII
Cross
VIII
Maternal plant
Paternal plant
Male sterility in 100% of offsprings
A
FRT
TapP
T7Polymerase
FRT
Lox
A
FRT
Lox
T7P
Toxin
Lox
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a
Hybrid cross
Recovery of fertility in 100% of hybrid offsprings
Or
A
FRT
TapP
T7Polymerase
FRT
Lox
a
A
FRT
Lox
T7P
Toxin
Lox
a

Fig. 2

To effect male sterility in a plant, the two transcribable polynucleotide sequences must be expressed such that when expressed cytostatic or cytotoxic activity only occurs in the stamen tissue or tissues (e.g., anther, pollen or tapetum).

Thus, according to a preferred embodiment of this aspect of the present invention, the second promoter is an stamen tissue-specific promoter. As used herein the term "stamen" refers to the male fertilizing organ of a flowering plant, including anther tissues. As used herein the phrase "anther tissues" or the term "anther" is meant to include the pollen and tapetum tissues as well.

In the case of promoter DNA sequences, stamen specific promoters are typically specific to the anther tissues and include regulatory sequences which direct the transcription of associated transcribable sequences so that the corresponding RNA is present in anther tissues in concentrations at least 100-fold that observed in other tissues.

Anther-specific promoters are well known in the art, examples of which include, but are not limited to, a tapetum-specific promoter such as the tobacco anther promoter, ant32, an anther-specific promoter such as that from LAT52 (Twell et al., Mol. Gen. Genet. 217: 240–245 (1989)); a pollen-specific promoter such as that from Zm13 (Guerrero et al., Mol. Gen. Genet. 224: 161–168 (1993)) or a microspore-preferred promoter such as that from apg (Twell et al., Sex. Plant Reprod. 6: 217–224 (1993). Additional examples are provided in, for example, U.S. Pat. No. 5,477, 002, which discloses promoter sequences from anther-specific genomic clones which are operatively linked to a DNA sequence coding for a desired polypeptide to enable anther specific expression.

By utilizing stamen-specific promoters, the resulting transgenic plants will express the transactivator only in the stamen tissues of the plant.

As already mentioned hereinabove, a transactivator expressed from the second transcribable polynucleotide sequence of an expression cassette can be an RNA polymerase, a transcriptional activator or a translational activator.

In a preferred embodiment of this aspect of the present invention the transactivator is a bacteriophage RNA polymerase such as, but not io limited to, a T7 RNA polymerase, a T3 RNA polymerase or an SP6 RNA polymerase.

As such the first promoter which drives the expression of the first transcribable polynucleotide sequence includes a sequence recognizable by bacteriophage RNA polymerase such that in the presence of the bacteriophage RNA polymerase and providing operational linkage between the first transcribable polynucleotide sequence and the first promoter, transcription of the first polynucleotide sequence is effected.

According to a preferred embodiment of this aspect of the invention the expression product of the first transcribable polynucleotide sequence is utilized to disrupt formation of viable pollen when expressed.

As mentioned hereinabove the resultant expression product of the first transcribable polynucleotide sequence can be either a polypeptide or an RNA molecule such as for example an anti-sense RNA molecule or a ribozyme RNA molecule.

Examples of polypeptides suitable for use in this aspect of the present invention include, but are not limited to, proteins capable of inhibiting the synthesis of macromolecules that are essential for cellular function, enzymes that degrade macromolecules that are essential for cellular function, proteins that alter the biosynthesis or metabolic metabolism of plant hormones and proteins that inhibit a specific function or development of anther/tapetum cells.

For example, Mariani et al., Nature, 347:737, (1990), have shown that expression of either *Aspergillus oryzae* RNase-T1 or an RNase of *Bacillus amyloliquefaciens*, designated "BARNASE", in the tapetal cells of a plant induced destruction of the tapetal cells, resulting in male sterility. Other genes can be used as alternatives to BARNASE for the development of male sterile plants, such as an anther-specific β-1,3-glucanase (Hird et al. The Plant Journal 4:1023–1033, 1993), or the male sterility gene described by Aarts et al. Nature 363: 715–717, 1993.

Additional polypeptides include diphtheria Toxin A-chain (DTA), which inhibits protein synthesis, Greenfield et al. (1983), Proc. Natl. Acad., Sci.:USA, 80:6853; Palmiter et al. (1987), Cell, 50:435; Pectate lyase pelE from *Erwinia chrysanthemi* EC16, which degrades pectin, causing cell lysis. Keen et al. (1986), J. Bacteriology, 168:595; T-urf13 (TURF-13) from cms-T maize mitochondrial genomes; this gene encodes a polypeptide designated URF13 which disrupts mitochondrial or plasma membranes. Braun et al. (1990), Plant Cell, 2:153; Dewey et al. (1987), Proc. Natl. Acad. Sci.:USA, 84:5374; Dewey et al. (1986), Cell, 44:439; Gin recombinase from phage Mu a gene, which encodes a site-specific DNA recombinase which will cause genome rearrangements and loss of cell viability when expressed in cells of plants. Maeser et al. (1991), Mol. Gen. Genet., 230:170–176; Indole acetic acid-lysine synthetase (iaaL) from *Pseudomonas syringae,* which encodes an enzyme that conjugates lysine to indole acetic acid (IAA). When expressed in the cells of plants, it causes altered developments due to the removal of IAA from the cell via conjugation. Romano et al. (1991), Genes and Development, 5:438–446; Spena et al., Mol. Gen. Genet., (1991), 227:205–212; Roberto et al. (1992 ~~XXX~~) Proc. Natl. Acad. Sci.:USA, 87:5795–5801; CytA toxin gene from *Bacillus thuringiensis Israeliensis* which encodes a protein that is mosquitocidal and hemolytic. When expressed in plant cells, it causes death of the cell due to disruption of the cell membrane. McLean et al. (1987), J. Bacteriology, 169:1017–1023; Ellar et al. (1990), U.S. Pat. No. 4,918,006; and, biotin binding proteins such as Streptavidin and avidin as further detailed in WO 96/40949 and 99/04023 which describe the use of a biotin binding protein for the induction of male sterility.

(1992)

In addition, the first polypeptide sequence can also encode DNAse, RNAse; protease; salicylate hydroxylase and the like.

In cases where it may be beneficial to target the polypeptide product expressed from the first transcribable polynucleotide sequence to a subcellular compartment such as the chloroplast, vacuole, peroxisome, glyoxysome, cell wall or mitochondrion, or for secretion into the apoplast, the first transcribable polynucleotide sequence includes a signal sequence, 5' and/or 3' to the region of the polypeptide encoding sequence. Targeting sequences at the 5' and/or 3' end of the structural gene may determine, during protein synthesis and processing, where the encoded protein is ultimately compartmentalized. The presence of a signal sequence directs a polypeptide to either an intracellular organelle or subcellular compartment or for secretion to the apoplast. Many sigsequences are known in the art. To this end, see, for example, Becker et al., Plant Mol. Biol. 20: 49 (1992), Close, P. S., Master's Thesis, Iowa State University (1993), Knox, C., et al., "Structure and Organization of Two Divergent Alpha-Amylase Genes From Barley", Plant Mol.

known in the art. Such methods are further described in the manual and text books listed in the preamble of the Examples section below.

Thus, the present invention provides methods and expression cassettes with which exogenic allelism can be generated in a non-human eukaryotic organism. Such allelism can be of great utility in cases where complete segregation of the allelic exogenes is desirable in subgenerations, such as the case of male sterile plants and their resultant male fertile hybrid progeny.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I–III Ausubel, R. M., ed. (1994); Cell Biology: A Laboratory Handbook" Volumes I–III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I–III Coligan J. E., ed. (1994); "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods and Enzymology" Vol. 1–317 Academic Press; all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

Example 1

Example 1 describes an expression cassette suitable for the generation of allelism in a plant, which allelism is used to generate 100% reversible male sterility. Since hybrid seed production is of commercial importance and since complete recovery of male fertility is crucial for high grain crop yields a system was developed which allows for the generation of male sterility but at the same time allows complete recovery of male fertility in progeny resultant from an outcross such as, for example, a hybrid outcross. As such this system can be used with high efficiency in hybrid seed production.

It will be appreciated that methods pertaining to plant self crossing and outcrossing utilized herein for the propagation of transformed plants and for subsequent hybrid seed production are commonly practiced in the art and as such no further description is necessary.

FIG. 1 outlines one method for generating reversible male sterile plants according to the teachings of the present invention.

Plant tissue is transformed with cassette I by, for example, an agrobacterium mediated gene transfer method and transgenic issue is regenerated into identical transgenic plants which harbor a single copy of cassette I. Cassette I includes a Tapetum specific promoter (TapP) such as ant32 or any other suitable stamen tissue specific promoter, followed by a LOX direct repeat. Following the LOX sequence is a T7 promoter region (T7P) which is recognized by the bacteriophage T7 polymerase which is not normally produced in plants. As such expression from the T7 promoter requires the presence of exogenous T7 polymerase gene or gene product. The T7 promoter is operatively linked to a cell cytotoxic molecule coding sequence designated as the toxin. In the presence of a T7 polymerase the toxin is expressed from the T7 promoter. A toxin which can be used in accordance with the present invention encodes a protein or an RNA molecule capable of disrupting the production of functional pollen cells or the formation of pollen as further detailed hereinabove. The toxin gene is followed by a second Lox site (direct repeat) which is followed by a T7 polymerase gene which is not linked to an operable promoter.

The transgenic plant including this cassette is crossed with an isogenic plant which is homozygous for a recombinase gene (CRE) expressible under a strong constitutive promoter such as the 35 S promoter but which does not contain cassette I. The transgenic plant including cassette I is also selfed to obtain a homozygote (III).

The plant resultant from cross II is selfed to obtain a plant containing cassette IV. Cassette IV results from the excision of the toxin and the T7 promoter regions. This excision brings the T7 polymerase region in close proximity to the TapP promoter region (separated by the Lox site which is 34 bp in length) such that the T7 polymerase gene is now under the transcriptional control of the TapP promoter. The plants harboring cassette IV will express the T7 polymerase in tapetal cells which should have no deleterious effects on pollen production. It will be appreciated in this case that since the direct repeat (Lox) site is immediately downstream of the TapP promoter it is important to either use direct repeats which do not contain an ATG codon therein or to eliminate any possible ATG start codon from the direct repeat sequence (using site specific mutation) such that transcription starts from the T7 polymerase ATG.

In the next step plant IV is crossed with plant III which does not express any of its exogenes, to yield a plant including a chromosome pair which is made up of a chromosome from plant III and a chromosome of plant IV. The resultant plant (V) is male sterile because the T7 polymerase produced in tapetal cells from chromosome A binds the T7 promoter region on chromosome A and drives the expression of the toxin which leads to the degeneration of the tapetal cells and to male sterility.

This plant serves as the maternal plant for subsequent hybrid crosses (VII) in which the pollen of a compatible non-transgenic male fertile plant (VI) is used to fertilize plant V to yield (from plant V) fertile offsprings. This is achieved because of the 100% segregation of chromosomes A and A. The seeds collected from plant V are hybrid seeds which include either cassette VIIa or cassette VIIb.

It will be appreciated that the specific arrangement of the genes, recombination sites and promoters in cassette I can be altered and yet produce very similar results. For example the TapP promoter can be linked to the T7 polymerase and both flanked by the Lox sites. In this case the T7 promoter can be separated from the toxin gene by the TapP promoter and the T7 polymerase. Following excision the toxin is brought under the control of the T7 promoter.

Example 2

An alternative method for generating allelism is shown in FIG. 2. Again this method is shown with specific reference to induced male sterility, it is to be understood however that this method can be applied to generate allelism for any purpose.

FIG. 2 outlines another method for generating reversible male sterile plants according to the teachings of the present invention.

Plant tissue is transformed with cassette I and transgenic tissue is regenerated into identical transgenic plants. These transgenic plants are male sterile because they express both the T7 polymerase and as a result the toxin expressed from the T7 promoter

According to this method cassette I includes a T7 polymerase operatively linked to the TapP promoter such as, for example, ant32, both flanked by the FRT recombinase sites. Cassette I further includes a toxin gene operatively linked to a T7 promoter, both flanked by the Lox sites.

Identical plants harboring cassette I are pollinated by a Cre recombinase plant (II) (homozygous for Cre) and an FLP recombinase plant (III) (homozygous for FLP). The resultant plants (IV and V) which contain either one of the gene and linked promoter are male fertile. As such these plants are selfed in order to lose the recombinase gene and to establish homozygotes of their recassettes (VI and VII). Plants VI and VII are crossed and the generated male sterile plant includes the two exogenes (T7 polymerase and the Toxin) in an allelic relationship. From this point on production of hybrid male fertile plants follows that described in Example 1.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

What is claimed is:

1. A method of generating a male sterile plant, the method comprising the steps of:

(a) providing a first plant and a second plant each comprising an expression cassette in the same chromosomal location, said expression cassette comprising:

(i) a first segment comprising a first transcribable polynucleotide sequence, said first transcribable polynucleotide sequence being operatively linked to a first promoter sequence, wherein said first segment is flanked by first site-specific recombination sequences; and

(ii) a second segment, being linked to said first segment, said second segment comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, wherein said second segment is flanked by second site-specific recombination sequences;

(b) introducing by transformation or crossing a first polynucleotide sequence encoding a first recombinase into said first plant, wherein said first recombinase recognizes the first site-specific recombination sequences so as to excise said first segment and produce a first plant comprising the first recombinase, selfing said first plant comprising the first recombinase, and selecting a progeny devoid of the first polynucleotide sequence encoding said first recombinase, wherein the progeny comprises the second segment of the expression cassette but not the first segment;

(c) introducing by transformation or crossing a second polynucleotide sequence encoding a second recombinase into said second plant, wherein said second recombinase recognizes the second site-specific recombination sequences so as to excise said second segment and produce a second plant comprising the second recombinase, selfing said second plant comprising the second recombinase, and selecting a progeny devoid of the second polynucleotide sequence encoding said second recombinase, wherein the progeny comprises the first segment of the expression cassette but not the second segment; and

(d) crossing the progeny resulting from step (b) with the progeny resulting from step (c), so as to generate an offspring plant characterized by exogenic allelism, wherein expression of the first and the second transcribable polynucleotide sequences results in male sterility of the plant.

2. The method of claim 1, wherein the first transcribable polynucleotide sequence encodes a cytotoxic polypeptide or a cytostatic polypeptide.

3. The method of claim 2, wherein the cytostatic or cytotoxic polypeptide is pectate lyase, 1-3 β-glucanase, avidin, streptavidin, diphtheria toxin A-chain, URF13, indole acetic acid-lysine synthetase, cytA toxin, RNase-TI or Barnase.

4. The method of claim 1, wherein the first transcribable polynucleotide sequence encodes an antisense RNA molecule.

5. The method of claim 1, wherein the second transcribable polynucleotide sequence encodes an expression product that transactivates the expression of the first transcribable polynucleotide sequence.

6. The method of claim 5, wherein the expression product is a bacterial RNA polymerase or a bacteriophage RNA polymerase.

7. The method of claim 1, wherein the second promoter sequence is selected from the group consisting of constitutive promoters and induced promoters.

8. The method of claim 1, wherein the second promoter sequence is a tissue specific promoter.

9. A plant or plant seed produced according to the method of claim 1, wherein the plant or the plant seed is characterized by exogenic allelism, and by a genome that lacks a polynucleotide sequence encoding an exogenic recombinase.

10. A male sterile plant heterozygous for an expression cassette comprising:

(a) a first segment comprising a first transcribable polynucleotide sequence, said first transcribable polynucleotide sequence being operatively linked to a first promoter sequence, wherein said first segment is flanked by first site-specific recombination sequences; and

(b) a second segment, being linked to said first segment, said second segment comprising a second transcribable polynucleotide sequence, said second transcribable polynucleotide sequence being operatively linked to a second promoter sequence, wherein said second segment is flanked by second site-specific recombination sequences, and wherein said second transcribable polynucleotide sequence encodes a polypeptide or an RNA molecule that regulates the expression level of a product of said first transcribable polynucleotide sequence,

wherein expression of the first and the second transcribable polynucleotide sequences results in male sterility of the plant.

* * * * *